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Use of a Dilute Aqueous Solution (5 mcg/ml) of a Benzimidazole Derivative with Potent Morphine-Like Actions Orally as a Presumptive Reinforcing Agent in Conditioning of Drug-Seeking Behavior in the Rat

By

Abraham Wikler, M.D.

Phillip C. Green

Herbert D. Smith

and

Frank T. Pescor

From the

National Institute of Mental Health

Addiction Research Center

U. S. Public Health Service

Lexington, Kentucky

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During the past 12 years, one of us has repeatedly called attention to certain aspects of the behavior of addicts which strongly suggest (a) that relapse after "cure" may represent, at least in part, a conditioned response to stimuli that have regularly been associated with the periodic relief of such abstinence distress as develops between doses of opiates during previous episodes of addiction (1,2,3,4); and (b) that the probability of such "conditioned" relapse is directly related to the "effort" ("hustling") expended by the addict in his drug-seeking behavior during previous episodes of addiction (5). For heuristic purposes, these concepts have been expressed formally in terms of both "classical" (Pavlovian) and "instrumental" (including "operant") models of conditioning (1,6,7,8), the former emphasizing the role that conditioning of the abstinence syndrome may play in relapse, and the latter, the role of the organism's manipulative activity.

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More recently, a few investigators, operating on hypotheses similar in some respects to those discussed above, have reported evidence obtained in studies on animals indicating both that the opiate abstinence syndrome can become conditioned (9) and that "preference" for morphine, persisting for periods beyond the expected duration of the abstinence syndrome, can be developed by instrumental conditioning during addiction (10,11).

In our laboratories, repeated attempts to condition either the morphine abstinence syndrome or drug-seeking behavior in rats have previously met with failure, apparently because of the aversive reactions induced by the pain of subcutaneous injection of morphine or the bitter taste of solutions of morphine or other opiates. Likewise, a promising study on rats in which the animals delivered doses of morphine to themselves through an implanted intraperitoneal catheter by an operant technique, ended disastrously after a few weeks, because the intraperitoneal opening of the polyethylene catheter became occluded by a thick membrane. Not to be deterred by such trifles, we began to consider seriously the preparation of animals with chronically implanted intravenous catheters, although the prospects for maintaining such preparations intact for several months were not bright.

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Meanwhile, however, our attention was directed to the properties of a new drug, 1-(Beta-diethylaminoethyl)-2-(p-ethoxybenzyl)-5-nitrobenzimidazole methane sulfonate (NIH-7607), which was then under study for its addiction liability on the research wards of this Center. This drug, which will be referred to by its ARC number, I-G-2, in this report, had been synthesized by chemists of the Ciba Pharmaceutical Company in Basel, Switzerland, and was found by their pharmacologists (12,13) and others subsequently to exert typical morphine-like actions in animals in extremely small doses. Thus, I-G-2 is 1,000 times more potent than morphine as an analgesic in rats (13), and 1,500 times in the mouse (14); in addicted monkeys, I-G-2 is 1,500 times as potent as morphine in suppressing signs of abstinence from morphine (15). In post-addicts, Isbell and Fraser (16) found that I-G-2 is 80-120 times more powerful than morphine as a euphoriant in single oral doses, and 1 mg of I-G-2 is as effective orally as 60 mg of morphine subcutaneously, in suppressing abstinence from morphine. Tolerance to repeated doses of I-G-2 develops rapidly in the rat (13) and in man (16), and in the latter, the degree of "physical dependence" developed is comparable in intensity with that produced by equivalent doses of morphine (16).

Such evidence of the morphine-like properties of I-G-2, and of its very much greater potency, suggested to us the possibility that rats might not be averse to drinking an aqueous solution of this drug, in concentrations that would produce morphine-like effects after consumption of small quantities. A "single-blind" taste-discrimination test performed by one of the authors on another, yielded a threshold concentration of 4-5 mcg of I-G-2 per ml of water. Hoping that the discriminative capacity of the rat is somewhere in this vicinity, calibrated drinking tubes filled with 3, 5 or 10 mcg/ml aqueous solution of I-G-2 were offered to rats deprived of water for 24 hours. The rats drank all the solutions avidly, and within four to seven minutes after beginning to drink (or after consumption of 66-144 mcg/kg of I-G-2) they exhibited morphine-like effects -- exophthalmos, tail rigidity, stupor (snout sometimes falling into the well of the drinking tube) and/or hyperactivity (quick, jerky movements of head, darting about, "exploring," chewing on floor of the cage). One rat, after consuming about 330 mcg/kg of I-G-2, lay prostrate on its side with outstretched hindfeet, breathing irregularly. Within a few minutes after subcutaneous injection of 10 mg/kg of nalorphine, however, it recovered, assuming the upright position, and responding to prodding. Addicted rats (maintained on

200 mg/kg of morphine by subcutaneous injection once daily for several months), likewise showed no apparent aversion to the I-G-2 solution (5 mcg/ml) when water-deprived. These animals were tested just prior to their regular daily dose of morphine, when the acute effects of the previous day's dose (tail rigidity, hyperactivity) were no longer apparent, and when they were exhibiting signs of morphine-abstinence, notably, repeated discrete twitches of the skin of the back, resembling the rapid skin-shaking of a wet dog, or of a horse shaking off a fly (see below). As in the non-addicted rats, typical morphine-like effects appeared within four to seven minutes after beginning to drink, and concomitantly, the "wet dog" twitches ceased, suggesting relief of abstinence, which persisted until the time for the regular daily dose of morphine a few hours later. The only difference observed in the effects of I-G-2 in the addicted rats was that the "sedative" effects were much shorter in duration, hyperactivity predominating within an hour after ingestion of the drug.

These preliminary observations indicated that a dilute solution of I-G-2 might well be substituted for morphine as a reinforcing agent by the oral route, and thereby enable us to circumvent the difficulties that attended our earlier efforts

to induce rats to drink solutions of morphine or other opiates, or to welcome subcutaneous injections of these materials. The present communication is in the nature of a "progress report" on two studies that have been initiated with this purpose in mind.

METHODS

In Study A, 6 "experimental" rats, gradually brought up to a constant daily dose level over a period of six to ten weeks, are maintained on once-daily subcutaneous injections of 200 mg/kg of morphine, given about 2 p.m., while 6 "control" rats receive volumetrically equivalent subcutaneous injections of physiological saline solution on the same schedule. All rats are allowed food ad libitum, but they are deprived of water for 22 hours each day, after the training and testing experiments in the morning. The study is designed to proceed in three phases. In Phase I, all rats are given access each morning to a wire cage 24 x 17.5 x 17.5 cm, divided into two equal compartments by a transparent vertical partition from the central entrance to which they can see two drinking tubes, placed vertically at the far end of each compartment. The rat can enter either compartment, drink from the tube therein, and also go back around the partition to the other compartment to drink from the tube there. On three successive days both tubes contain water, but on the

fourth day, one of the tubes contains a 5 mcg/ml solution of I-G-2, the compartment in which this tube is placed being alternated in successive four-day blocks (total of 17 blocks, or 68 days). Discriminative stimuli (blinking lights, metal strips with alternating black and white stripes on drinking tube and floor), for later use in Phase II, are also provided, but in Phase I, the sides of the compartment in which they are placed are randomized. Each day, the rats are allowed to drink for 15 minutes, and records are made of the volumes consumed from each tube every day, during successive three-minute periods. After each 15-minute trial, the rats are returned to their home cages, where they are allowed to drink water ad libitum from a metal cup for two hours, after which all water is removed until the next morning. The purposes of Phase I are to determine whether or not, <u>without discriminative training</u>, either the "experimental" or the "control" rats exhibit preference for, or aversion to I-G-2 in the concentration used on the basis of <u>taste</u>, and whether or not the discriminative stimuli have appetitive or aversive properties independent of I-G-2. Since the grossly visible effects of I-G-2 begin as early as four minutes after commencement of drinking, the critical measures for taste discrimination are the relative quantities of I-G-2 solution and tap water drunk from a given tube (right or left)

during the first three minutes. Similarly, the critical measures for the reinforcing properties of the discriminative stimuli per se are the relative amounts of water (only) consumed when these stimuli are in the right or left compartment. In Phase I, the I-G-2 solution is presented only every fourth day, to minimize conditioning by effect and the development of tolerance. In Phase II, all the rats, maintained on morphine or saline and on food-and-water schedules as before, are given access to a cage similar to that already described but constructed of plastic and of larger dimensions (40 x 26 x 35 cm) with an opaque partition. However, the daily schedule is designed to permit learning of a discrimination of the I-G-2 solution by effect, using successive six-day block trials as follows. On the first four days of each block, only one tube is presented, containing either tap water the first and second days, or the I-G-2 solution on the third and fourth days, the order being reversed for each successive block. The compartment in which the I-G-2 solution is placed is always equipped with the discriminative stimuli previously mentioned, and remains the same for a given rat throughout all the blocks, though initially, the rats are assigned to one or the other I-G-2 compartment (right or left) in alternation. Also, after

commencing to drink on each of the four days, egress from the compartment is prevented by closing a rear gate, to ensure that whatever reinforcing effects the water or I-G-2 solution may have, will be associated with that compartment and its discriminative stimuli (if any) only. On the fifth day, the water tube is invariably presented in its usual compartment, and egress is prevented as on the previous four days. On the sixth day of each block, both water and I-G-2 tubes (the latter with the discriminative stimuli) are presented in their respective compartments, the egress gates remain open, and the rats are permitted to drink from either tube at any time within the 15-minute period of testing. The critical measure in Phase II is the relative amounts of I-G-2 solution and water consumed in the first three minutes by the "experimental" and "control" rats on each of the sixth-day test trials, after correction for whatever appetitive or aversive effects the discriminative stimuli may have been found to exert per se in Phase I.

In Phase III, all rats will be tested for "relapse" several weeks after abrupt withdrawal of morphine, under conditions of water satiation and water-deprivation, by the technic described for the sixth day of each block in Phase II.

In Study B, "experimental" and "control" rats are prepared and maintained on morphine or saline injections respectively as in Study A. Each morning (about 18 hours after the previous daily dose of morphine, 200 mg/kg), they are placed in an operant conditioning apparatus equipped with a lever which, when depressed, activates a mechanism that uncovers a dipper containing 0.25 ml of fluid, set on the floor of cage about 6 cm to the rear of the lever. No trials are run on Saturday or Sunday (although, the daily morphine or saline injections are continued), but on the other five days of each week, the rats are given access to the lever under four conditions which are randomized and balanced over a period of seven weeks: 22 hours water deprivation, water reinforcement; 22 hours water deprivation, I-G-2 solution (5 mcg/ml) reinforcement; water satiation, water reinforcement; water satiation, I-G-2 reinforcement. Each trial in the operant conditioning apparatus proceeds for 15 minutes, after which water is supplied ad libitum in the home cages for two hours on days preceding the "water deprivation" runs, and throughout the day and night before the "water satiation" runs, food being available at all times throughout. Discriminative stimuli in the operant conditioning apparatus consist of rough (hardware cloth) surfaces for the floor and lever on trial days with water

reinforcement, and smooth (plastic) surfaces with I-G-2 reinforcement. In addition, on days with I-G-2 reinforcement, each lever press actuates a clicker placed inside the Skinner Box, to serve as a "secondary reinforcer" (representing the traditional "bad associate" of narcotic addicts' lore).

The critical measures in this study are the comparative rates of bar-pressing throughout the 15-minute trial periods within each group ("experimental" and "control") for water versus I-G-2 reinforcement, and comparative rates of bar-pressing between the two groups for water or I-G-2 reinforcement, both measures referring to water-satiation days. In addition, similar comparisons are made of unreinforced bar-pressing rates on water satiation days, since both "experimental" and "control" animals engage in such activity (possibly because of uncontrolled secondary reinforcers) as well. Study B is also scheduled to proceed in three phases, the first of which has already been described. In the second phase, bar-pressing rates for water and I-G-2 reinforcements will be compared within and between both groups of animals under conditions of water satiation during the acute morphine withdrawal period, and later, in the third phase, tests for "relapse" will be made in both groups, under conditions of water deprivation and water satiation, and the discriminative stimuli and (controlled) secondary reinforcer already described.

If positive results are obtained after completion of Study B, it is planned to conduct a similarly designed investigation, with a number of schedules of reinforcement, to analyze the influence of various parameters of "effort" or "hustling" on the probability of "relapse."

RESULTS

At the present time, only the first phases of both studies have been completed. The data for this phase of Study A have been analyzed only with regard to the question of the capacity of the animals to discriminate between water and the 1-G-2 solution by taste. As shown in Table 1, the overall differences obtained seem to indicate that, without discriminative training, the "experimental" rats exhibit a slight "preference" and the "control" rats a slight "aversion" to the 1-G-2 solution, but these differences are not statistically significant by the chi square test.

Several analyses were made of the data obtained in the first phase of Study B. In Table 2 are shown intergroup comparisons of "wet dog" responses per 15-minute trial in experimental and control animals on separate days, under the four conditions of the experiment. It is noteworthy that on water satiation days, the number of "wet dog" responses

exhibited by the experimental animals was greater than those of the control animals to highly significant degrees by the Mann-Whitney U test for intergroup comparisons (17), except for one trial day although, even then, the trend was in the same direction (mean of 5.20 for the experimental, and 2.60 for the control animals). In contrast, no significant differences were observed on water deprivation days regardless of whether the reinforcement used was water or the I-G-2 solution.

The analyses presented in Table 3[1] reveal that on water deprivation days, the reinforced bar-pressing rates of experimental rats exceeded those of control rats, regardless of whether water or I-G-2 was used as the reinforcement. On water satiation days, however, the reinforced bar-pressing rates of experimental animals generally exceeded those of control animals only when reinforcement was provided by I-G-2. Adding perhaps to the theoretical significance of these results are the comparisons (Table 3) between reinforced and non-reinforced bar-pressing rates of experimental and control animals on water satiation days. When reinforced by I-G-2, the reinforced bar-pressing rates are significantly different for the two groups of animals, but not the non-reinforced rates; when reinforced by water, neither the reinforced nor the non-reinforced rates are generally different to significant degrees. On the other

hand, the intragroup comparisons shown in Table 4[1] indicate that on water satiation days, neither the experimental nor the control groups showed significant differences between bar-pressing rates with water and I-G-2 reinforcements, while on water deprivation days both groups exhibited significantly greater rates with water than with I-G-2 reinforcement.

DISCUSSION

The data for the first phase of Study A indicate that, without discriminative training, neither experimental nor control animals display either preference for or aversion to a 5-mcg/ml solution of I-G-2. This finding is of basic importance for the subsequent phases of both studies, since the hypotheses to be tested predict the development of a "preference" for I-G-2 by the experimental, but not the control animals.

The findings so far obtained in Study B are of interest in several respects. The much greater incidence of "wet dog" responses in the experimental rats, observed at 18 hours of abstinence and up to the time of the regular daily dose of morphine, as well as the virtual disappearance of these responses after injection of morphine or ingestion of I-G-2, suggest that they may be reliable indicators of the morphine abstinence

syndrome in this species. Earlier observations in this laboratory of a more casual sort, indicate that "wet dogging" continues at a high rate for two or three days after abrupt withdrawal of morphine and then gradually subsides to incidences exhibited by nonaddicted rats over a period of about two to three weeks. However, more quantitative studies are needed to establish the reliability of this measure, and to correlate it with other abstinence phenomena in these animals. Also, the curious finding that water deprivation reduces the incidence of "wet dog" responses in acutely abstinent rats to those of control animals, requires elucidation.

The generally higher bar-pressing rates of experimental than control animals in Study 5 may be due to factors other than that postulated theoretically -- namely, that "physical dependence" provides a "drive" state through reduction of which, "successful" drug seeking behavior becomes reinforced. Seemingly, the operation of such other factors is suggested by the data obtained on water deprivation days, when the bar-pressing rates with water reinforcement exceeded those with I-G-2 reinforcement, not only in the control but also in the experimental animals. This, however, may be due to the fact that on water deprivation days, both groups of animals

consume enough of the I-G-2 solution to produce visible morphine-like effects within four to seven minutes, after which their rates of bar pressing declines sharply. On water satiation days, bar-pressing rates were much lower in both groups, and generally, neither group consumed enough I-G-2 to affect the rates of bar pressing. Under such circumstances, certain of the evidence (Table 3) indicates that the I-G-2 solution was reinforcing for the experimental group only, but evidence for a neat discrimination between water and I-G-2 reinforcement conditions by these animals was not obtained in this phase of the study (Table 4). As noted earlier, both of these studies are still in progress and it is hoped that more conclusive results, either consonant or not consonant with the hypothesis, will be forthcoming in the near future.

SUMMARY

1. Evidence in the literature is reviewed supporting the concept that, in part at least, relapse is due to conditioning factors, both of the "classical" and "instrumental" variety operating during previous episodes of addiction to narcotic drugs.

2. The morphine-like properties of a new benzimidazole derivative (NIH-7607, ARC I-G-2) are described and evidence is presented that neither morphine addicted nor control rats display either preference for or aversion to a 5-mcg/ml aqueous solution of the drug, without discriminative training.

3. Two studies in progress are described, in which this concentration of I-G-2 is being used as a reinforcing agent in attempts to condition drug-seeking behavior in rats.

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FOOTNOTE 1

For computation of intergroup and intragroup differences in bar-pressing rates (Tables 3 and 4), only the data obtained during the last 18 days of Phase I (Study B) were used, since just previously, a change had been made in the fluid-reinforcement dispensing mechanism, which resulted in augmentation of the rates of bar pressing of all animals, "experimental" and "control."

Table 1.

Comparisons of per cent fluid consumed in first three minutes of drinking from right and left tubes, when these contained I-G-2 solution (8 trials for right, 9 for left), and when both tubes contained tap water (24 trials for right, 27 for left).

	Experimentals (N = 3 to 6)			Controls (N = 6)		
	Tube-Locations		D	Tube-Locations		D
Per Cent Fluid Consumed	I-G-2 on right	Both H_2O		I-G-2 on right	Both H_2O	
From Right	76.2	66.1	10.1	78.6	77.2	-1.4
	I-G-2 on left	Both H_2O		I-G-2 on left	Both H_2O	
From Left	54.8	61.9	-7.1	21.7	26.1	-4.4
	Mean Difference		+3.0	Mean Difference		-3.0

The plus sign (+) indicates consumption of an excess of I-G-2 over water.

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Table 2.

Mean "Wet Dog" Frequencies ($\bar{f}$). Intergroup Comparisons Between Experimental (E) and Control (C) Animals.

Condition	Trial No.	Experimentals		Controls		$\bar{f}$ Diff.	Total Mann-Whitney U Ranks		Sig. Level
		N	$\bar{f}$	N	$\bar{f}$		E	C	
WSI	11	5	9.80	5	1.80	8.00	35.5	19.5	*
	13	5	11.20	5	1.40	9.80	40.0	15.0	***
	18	4	16.00	5	2.40	13.60	30.0	15.0	**
WSW	8	5	5.20	5	2.60	2.60	34.5	20.5	N.S.
	9	5	10.60	5	1.60	9.00	38.0	17.0	**
	14	5	15.60	5	1.80	13.80	40.0	15.0	***
	17	4	9.75	5	2.40	7.35	30.0	15.0	**
WDI	12	5	2.00	5	0.80	1.20	34.0	21.0	N.S.
	15	4	3.75	5	1.00	2.75	21.0	24.0	N.S.
WDW	7	5	2.80	5	2.40	0.40	30.5	24.5	N.S.
	10	5	4.60	5	4.20	0.40	34.0	21.0	N.S.
	16	4	4.75	5	2.20	2.55	26.0	19.0	N.S.

CONDITIONS:

WDW Water deprivation, water reinforcement

WDI Water deprivation, I-G-2 reinforcement

WSW Water satiation, water reinforcement

WSI Water satiation, I-G-2 reinforcement

* $P \leq .05$

** $P \leq .01$

*** $P \leq .001$

N.S. Not significant

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Table 3.

Mean Bar-Pressing Frequencies ($\bar{f}$). Intergroup Comparisons Between Experimental (E) and Control (C) Animals.

Condition	Trial No.	N of Ss E	N of Ss C	REINFORCED $\bar{f}$ E	$\bar{f}$ C	$\bar{f}$ Diff.	Mann-Whitney U Rank E	Mann-Whitney U Rank C	Sig. Level	UNREINFORCED $\bar{f}$ E	$\bar{f}$ C	$\bar{f}$ Diff.	Mann-Whitney U Rank E	Mann-Whitney U Rank C	Sig. Level
WSI	4	5	5	9.00	1.80	7.20	39.0	16.0	**	2.80	3.80	-1.00	25.0	30.0	N.S.
	6	5	5	4.00	0.00	4.00	37.5	17.5	**	4.00	3.60	0.40	28.0	27.0	N.S.
	11	5	5	15.00	0.60	14.40	39.5	15.5	**	15.00	6.20	8.80	34.0	21.0	N.S.
	13	5	5	4.00	2.00	2.00	35.5	19.5	*	4.60	5.20	-0.60	29.0	26.0	N.S.
	18	4	5	4.75	2.20	2.25	21.0	24.0	N.S.	9.00	3.20	5.80	25.5	19.5	N.S.
WSW	3	5	5	4.60	3.40	1.20	32.0	23.0	N.S.	8.20	3.20	5.00	28.5	26.5	N.S.
	8	5	5	6.80	1.40	5.40	36.5	18.5	*	15.20	4.40	10.80	31.0	24.0	N.S.
	9	5	5	5.60	1.00	4.60	29.5	25.5	N.S.	11.80	3.40	8.40	34.0	21.0	N.S.
	14	5	5	2.20	1.20	1.00	33.0	22.0	N.S.	2.60	3.20	-0.60	26.0	29.0	N.S.
	17	4	5	5.75	4.60	1.15	23.0	22.0	N.S.	2.25	3.00	-0.75	17.5	27.5	*
WDI	2	5	5	37.20	18.40	18.80	39.5	15.0	***	7.60	2.20	5.40	36.5	18.5	*
	5	5	5	45.20	21.20	24.00	39.5	15.5	**	21.20	6.00	15.20	37.0	18.0	**
	12	5	5	39.20	16.20	23.00	39.5	15.5	**	15.80	1.20	14.60	34.5	20.5	N.S.
	15	4	5	32.50	20.60	11.90	25.5	19.5	*	11.25	4.40	6.85	25.0	20.0	N.S.
WDW	1	5	5	48.80	31.80	17.00	36.0	19.0	*	27.80	3.00	24.80	37.5	17.5	**
	7	5	5	63.40	30.80	32.60	40.0	15.0	***	49.60	1.40	48.20	40.0	15.0	***
	10	5	5	62.40	30.80	31.60	40.0	15.0	***	37.20	3.20	34.40	37.0	18.0	**
	16	4	5	47.75	34.00	13.75	22.5	22.5	N.S.	51.75	4.20	47.55	23.5	21.5	N.S.

Conditions:

WDW Water deprivation, water reinforcement
WDI Water deprivation, 1-3-2 reinforcement
WSW Water satiation, water reinforcement
WSI Water satiation, 1-0-2 reinforcement

* $P \leq .05$
** $P \leq .01$
*** $P \leq .001$
N.S. Not significant

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Table 4.

Mean Reinforced Bar-Pressing Frequencies ($\bar{f}$). Intragroup Comparisons Between Experimental and Control Animals.

Condition	EXPERIMENTALS (N = 5)					CONTROLS (N = 5)				
	Reinforcement		Diff.	Wilcoxon T	Sig. Level	Reinforcement		Diff.	Wilcoxon T	Sig. Level
	I-G-2	Water				I-G-2	Water			
Water Satiation	7.61	4.76	2.85	2	N.S.	1.00	2.32	- 1.32	5	N.S.
Water Deprivation	38.48	56.03	-17.55	0	*	19.00	31.85	-12.85	0	*

* $P \leq .05$

N.S. Not significant